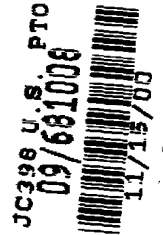


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APPARATUS FOR OPTICAL SCANNING OF MULTIPLE SPECIMENS

First Named Inventor: Dr. Johann Engelhardt

SUBMITTED BY

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specification	5056sp.xml
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Large Entity

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BASIC FILING FEE

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Utility Filing Fee	101	\$ 710

Subtotal For Basic Filing Fee: \$ 710

EXTRA CLAIM FEES

	Fee Code	Fee	Extra Claims	Fee Paid
Total Claims: 23	103	\$ 18	3	\$ 54
Independent Claims: 1	102	\$ 80	0	\$ 0

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SPECIFICATION

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APPARATUS FOR OPTICAL SCANNING OF MULTIPLE SPECIMENS

Cross Reference to Related Applications

This invention claims priority of a German patent application DE-199 57 413.8 which is incorporated by reference herein.

Background of Invention

- [0001] The present invention concerns an apparatus for optical scanning of multiple specimens, the specimens being associated with a specimen receiving device and being optically scannable by a scanning device. In addition to that the invention concerns as well with an apparatus for scanning multiple specimen regions on a large specimen.
- [0002] Arrangements of the generic type are used in particular for the detection of "micro-array biochips" (MABs). MABs are used in medical diagnostics, where a large number of MABs need to be examined and evaluated in a brief period of time. Specimen holders on which a large number of specific detection regions ("spots") are placed, preferably in a grid pattern, are among the devices that can be used as MABs. These detection regions usually have a diameter of approximately 50 to 100 μm and need to be examined in terms of their fluorescence properties. The spots are distributed on a specimen holder over a specimen field of up to 22 x 60 mm.
- [0003] Specimens distributed over such large object fields cannot be simultaneously imaged with a conventional confocal laser scanning microscope (CLSM), however, since the object fields of microscope objectives suitable for such applications are

too small for the purpose. The use of larger objectives or lenses, with a beam that is moved relative to the lenses, results in image irregularities and intensity fluctuations. The image irregularities are caused by residual or imaging errors in the lenses used, and the intensity fluctuations by partial contamination of the optical components in the beam path.

[0004] In addition, the illumination beam path of CLSMs is configured in such a way that the diffraction-limited scanning beam generally has, a diameter in the specimen plane in the range of 1–2 μ m. As a result, the specimens are scanned with a spatial resolution that is unnecessary and much too high for these applications. Laboratory use, in particular, demands a high throughput of specimen holders for examination, which is not attainable with presently known CLSMs. The operation of CLSMs is moreover complex, and requires considerable operating experience on the part of the user.

Summary of Invention

[0005]

[0006] It is therefore the object of the present invention to describe an apparatus for automatic optical scanning of multiple specimens and/or specimen regions, with which data recording from multiple specimens in a short period of time is possible. In this context, the optical system components are intended to be economical and of simple design. The entire system should be easy to use.

[0007] The aforesaid object is achieved by an apparatus which comprises a specimen receiving device for holding the specimens, a scanning device provided for optically scanning the specimens, the specimen receiving device defines an axis of rotation and the scanning device is arranged movably relative to the specimen receiving device.

[0008] What has been recognized according to the present invention is firstly that data recording from many specimens or specimen regions in a short period of time can be achieved by way of a rotating specimen receiving device. In particular, the rotary motion of the specimen receiving device advantageously makes it possible to

optically scan the same specimen several times, so as thereby, for example, to be able to compare multiple measurement results from the same specimen to one another. This is important principally for statistical evaluation of the measured specimens. For data recording from many specimens in the shortest possible time, the specimen receiving device can be of correspondingly large dimensions so that as many specimens or specimen holders as possible can be detected in one measurement pass.

- [0009] The specimen receiving device could rotate in opposite directions. For example, after the specimens have been optically scanned once, the specimen receiving device could be rotated in the opposite direction. It is also conceivable for the specimen receiving device to rotate in only one direction, preferably continuously.
- [0010] In a preferred embodiment, the scanning device is arranged movably relative to the specimen receiving device. This advantageously allows the entire region of the specimen receiving device to be optically scanned. For this purpose, the scanning device or the specimen receiving device could be linearly displaceable. This displacement or relative motion between scanning device and specimen receiving device could occur in the radial direction. The linear relative motion could also proceed along a secant.
- [0011] In a further embodiment, the scanning device is arranged rotatably or pivotably about a further axis, so that the relative motion between scanning device and specimen receiving device is accomplished by way of a rotation of the scanning device. A rotation of the specimen receiving device about a further axis is also conceivable if the scanning device is arranged in stationary fashion.
- [0012] In additionally advantageous fashion, the optical distance between the specimen and the scanning device is substantially constant in the context of a relative motion between the scanning device and the specimen receiving device. The principal result of this is to ensure that a defined specimen region is optically scannable, namely preferably the region in which the specimens to be scanned are in fact located. The condition that the optical distance between the specimen

receiving device and the scanning device remain substantially constant during a relative motion can be achieved by way of a corresponding arrangement of the axis of the specimen receiving device relative to the direction of motion of the scanning device. If the relative motion between the scanning device and the specimen receiving device is accomplished by rotation or pivoting of the scanning device about a further axis, the requirement for a constant optical distance between the specimen receiving device and the scanning device can be met by a substantially parallel arrangement of the two rotation or pivot axes. In particular when focused scanning light is used, the aforementioned features ensure that the specimens to be scanned are always, when viewed from the scanning device, located in the same position in the optical beam path.

[0013] In the context of an alternative embodiment, the specimen receiving device has a constant rotation speed. In particular, the rotation speed of the specimen receiving device is kept constant during optical scanning or data recording. The rotation speed could have a value that lies in a range from one to three hundred revolutions per minute.

[0014] It is also conceivable for the specimen receiving device to have a variable rotation speed. In this context, the rotation speed could be dependent on the relative position between the specimen receiving device and the scanning device. The region to be scanned that is close to the axis of the specimen receiving device in the radial direction supplies less specimen information per revolution than a region that is farther away from the axis of the specimen receiving device, since the circumference scanned with each revolution changes. A modifiable rotation speed could advantageously make allowances for this situation, namely the fact that the rotation speed is dependent on the detected data stream of the scanning device. The specimen receiving device could consequently have a steadily increasing rotation speed, while optical scanning by the scanning device is accomplished radially outward from a region close to the axis of the specimen receiving device.

[0015] The specimen receiving device can have a large mass. This is advantageous in

particular when the specimen receiving device rotates at a constant rotation speed. To prevent or minimize any rotational imbalance of the specimen receiving device, the latter is configured in rotationally symmetrical fashion with uniform mass distribution.

[0016] Concretely, the specimen receiving device could be configured so that it can receive different inserts. It would be conceivable, for example, for the specimen receiving device to receive a single, replaceable specimen vessel. This specimen vessel could be a conventional Petri dish or a culture vessel.

[0017] The specimen receiving device could also have a replaceable carousel insert. This carousel insert could receive individual specimen holders within it. Suitable specimen holders include conventional glass specimen holders that are usual in microscopy. Other specimen holders, for example from the field of MABs, are also usable. The individual specimen holders are arranged in the carousel insert with single-point symmetry with respect to the rotation center of the specimen receiving device, so that no imbalance occurs as the specimen receiving device rotates about its rotation axis.

[0018] Advantageously, the individual specimen holders are positionable in a predefinable plane in the carousel insert with retaining means. Concretely, the individual specimen holders are positioned resiliently or with spring force. This spring force presses the specimen holder against the retaining means. With this feature, a carousel insert can be loaded in the shortest possible time with specimen holders, including, in particular, in routine operation. This also ensures that all the specimen holders are positioned in one predefinable plane.

[0019] With regard to the statistical analysis of a plurality of specimen holders, the individual specimen holders are equipped with a specific identifier. This identifier could be configured, for example, in the form of a barcode, associated with which is information concerning the relevant experiment, the measurement series, or an assignment to a patient.

[0020] According to a further embodiment, autofocusing of the specimens is

provided. Autofocusing can be accomplished by way of a corresponding focusing optical system that is arranged in the scanning device. Corresponding positioning of the scanning device relative to the specimen receiving device would also be conceivable. Autofocusing of the specimens can ensure that all the specimens to be scanned are in fact imaged in focus. Autofocusing of the specimens could be implemented using known methods. Concretely, autofocusing could be performed using the triangulation method.

[0021] In the context of a further embodiment, the surface of the rotating specimen receiving device has a deviation in the direction of the optical axis of the scanning device that is preferably less than 20 μ m. Alternatively, the surface of the specimen vessel or of the specimen holder located in the carousel insert has a maximum deviation in the direction of the optical axis of the scanning device that also is preferably less than 20 μ m. As a result of this feature it would be possible to dispense with an autofocusing device, thereby simplifying data recording and allowing equipment costs to be reduced.

[0022] The specimens are scannable with at least one laser beam. The corresponding measurement signals are detected with at least one detector. In this context, the laser beam could scan in at least one direction relative to the scanning device. Alternatively, the laser beam could be stationary relative to the scanning device. A laser beam that is stationary relative to the scanning device is to be preferred to a scanning laser beam in terms of simpler alignment and cost-reducing production.

[0023] In a further embodiment, scanning is accomplished with laser light of different wavelengths. This is of considerable advantage in particular when specimens specifically marked with different fluorescent dyes are to be scanned. In this case the fluorescence signals could be detected with multiple detectors.

[0024] If multiple laser beams are used simultaneously, the beam path of the scanning device could be configured such that each laser beam illuminates a different specimen region. As an alternative to this, in particular when multiple laser beams of different wavelengths are used, all the laser beams could illuminate the same specimen region.

[0025] In additionally advantageous fashion, the axial extent of the focus region of the laser beam in the specimen region is less than 40 μ m. In this respect as well it is possible to dispense with the use of an autofocus system if the maximum deviation of the surface of the rotating specimen region always has a maximum deviation *[sic]* in the direction of the optical axis that is less than 20 μ m. With suitable axial positioning of the scanning device, all the specimens associated with the specimen receiving device are moved through the focus region of the scanning device during the entire data recording operation.

[0026] In terms of the size of the specimens to be examined or scanned, the lateral extent of the focus region of the laser beam in the specimen region is between 5 μ m and 200 μ m. Ultimately the lateral extent of the focus region of the laser beam in the specimen region must be selected in such a way that the scanning theorem is satisfied in terms of the average size of the specimens being scanned. The diameter of the laser focus in the lateral direction in the specimen region must thus be less than or equal to half the average specimen diameter.

[0027] The axial and/or lateral extent of the focus region of the laser beam in the specimen region can be adjusted by way of the optical beam path within the scanning device. In particular, the optical beam path of the scanning device can be of confocal configuration.

[0028] The incidence angle of the laser beam onto the surface of the specimen receiving device, the specimen vessel, or the specimen holder is selected in such a way that it differs from zero. This advantageously makes it possible to suppress the principal return reflection of the exciting light resulting, for example, from the transition between specimen holder and air or to block it out from the excitation and detection beam path. This is important in particular for the detection of fluorescent light, since it is then possible to use a (bandpass) blocking filter of lower strength, and the corresponding detector can thus detect the fluorescent light more effectively. An incidence angle for the laser beam differing from zero can be implemented by the fact that either the entire optical arrangement in the scanning device, or only a single mirror, is correspondingly mounted or aligned.

What must ultimately be ensured is that the laser beam striking the surface of the specimen receiving device, the specimen vessel, or the specimen holder is at a corresponding angle relative to the axis of the specimen receiving device.

[0029] In the interest of unequivocal data analysis, the specimen receiving device, the specimen vessel, or the carousel insert has synchronization markings. These could, for example, be detected by a photoelectric barrier. This makes possible an unequivocal association between the scanned image data and the corresponding positions of the specimen receiving device.

[0030] According to a further embodiment, provision is made for a back-transformation of the measured image data, present in cylindrical coordinates, into Cartesian coordinates. The information of the synchronization markings of the specimen receiving device, the specimen vessel, or the carousel insert is utilized for this purpose as well.

Brief Description of Drawings

[0031] There are various ways of advantageously embodying and developing the teaching of the present invention. Reference is made to the explanation below of exemplary embodiments of the invention with reference to the drawings. In conjunction with the explanation of the preferred exemplary embodiments of the invention with reference to the drawings, a general explanation is also given of preferred embodiments and developments of the teaching. In the drawings:

[0032] FIG. 1 shows, in a schematic general depiction, an exemplary embodiment of an apparatus according to the present invention for optical scanning of multiple specimens;

[0033] FIG. 2 schematically shows, in a schematic plan view, a carousel insert of the apparatus according to the present invention for optical scanning of multiple specimens; and

[0034] FIG. 3 shows, in a schematic side depiction, a carousel insert with specimen holders positioned therein.

Detailed Description

- [0035] FIG. 1 shows an apparatus for optical scanning of multiple specimens 1 and/or specimen regions, the specimens being associated with a specimen receiving device 2 and optically scannable by a scanning device 3.
- [0036] According to the present invention, specimen receiving device 2 is rotatable in one direction about an axis 4. It has to be understood that the specimen receiving device (2) is rotatable as well in opposite directions about the axis 4. Specimen receiving device 2 is guided during its rotation by pivot bearing rod 22. Scanning device 3 is linearly displaceable as shown by reference character 5, specifically in a radial direction. As an alternative to this, specimen receiving device 2 could also be displaceable in direction 6. Scanning device 3 could be arranged rotatably or pivotably about a further axis 7, guided in this case by a pivot bearing rod 23.
- [0037] During the linear displacement of scanning device 3 in direction 5, the optical distance between specimen 1 and scanning device 3 remains substantially constant, since displacement direction 5 is arranged perpendicular to axis 4.
- [0038] Specimen receiving device 2 has a constant rotation speed during data recording. A variable rotation speed of the specimen receiving device (2) is possible as well. Specimen receiving device 2 is halted only during specimen loading. Specimen receiving device 2 moreover has a large mass, is of rotationally symmetrical configuration, and receives a replaceable carousel insert 8.
- [0039] FIG. 2 shows a carousel insert 8 that receives individual specimen holders 9. It is evident from FIG. 3 that the individual specimen holders 9 are positionable in a definable plane on carousel insert 8 with retaining means 10. The individual specimen holders 9 are pressed with springs 11 upward against retaining means 10. In this context, the contact surface of specimen holder 9 against retaining means 10 defines the plane in which the individual specimen holders are to be positioned. The surface of the rotating specimen receiving device 2 and the surfaces of specimen holders 9 present in the carousel insert always have a maximum deviation, in the direction of optical axis 12 of scanning device 3, that is less than 20 μ m. Specimen receiving device 2 and carousel insert 8 are rotary

parts that are manufactured with a corresponding precision-engineered accuracy. In particular, the arrangement of holding means 10 on carousel insert 8 is fabricated with corresponding accuracy. Each specimen holders 9 may be provided with a specific identifier. The specific identifier of the specimen holders 9 is configured in the form of a barcode.

[0040] Specimens 1 are scanned with a laser beam from laser 13. The detected light of specimens 1 is detected with a detector 14. The illumination and detection beam is stationary relative to scanning device 3. The laser is an argon-krypton (ArKr) laser, which emits laser light of different wavelengths. The optical elements in the beam path of scanning device 3 are selected such that the axial extent of the focus region of the laser beam in specimen region 1 is smaller than $40\text{ }\mu\text{m}$, and the lateral extent is approximately $25\text{ }\mu\text{m}$. The optical beam path in scanning device 3 is of confocal configuration. Excitation pinhole 15 following laser 13 functions as a point illumination light source. Detection pinhole 16 preceding detector 14 functions as a detection aperture stop. The entire optical beam path of scanning device 3 is arranged in such a way that the incidence angle of the laser beam onto the surface of specimen holders 9 located in carousel insert 8 is 10 degrees, which is evident in schematic fashion from FIG. 1. Alternatively, only mirror 20 in conjunction with lens 21 could be arranged in such a way that the incidence angle equals 10 degrees. Because of the 10-degree incidence angle, principal reflection 17 occurring at the air/glass transition of specimen holders 9 is reflected out of the illumination and detection beam path. As a result, dichroic beam splitter 18 can be selected on the basis of its wavelength characteristic in terms of the fluorescent light to be detected, specifically so that undesired components of the principal reflection of the illuminating light from the air/glass transition do not need to be specially filtered out.

[0041] Carousel insert 8 shown in FIG. 2 has synchronization markings 19 that are detected by a photoelectric barrier (not depicted). The synchronization signals, in conjunction with the position signal of scanning device 3 moving in linear direction 5, make possible an association between the measured specimen detection signal and the corresponding local coordinates referred to carousel insert 8.

Claims

- [c1] 1. An apparatus for optical scanning of multiple specimens (1), comprises a specimen receiving device (2) for holding the specimens (1), a scanning device (3) provided for optically scanning the specimens, the specimen receiving device (2) defines an axis (4) of rotation and the scanning device (3) is arranged movably relative to the specimen receiving device (2).
- [c2] 2. The apparatus as defined in Claim 1, characterized in that the scanning device (3) or the specimen receiving device (2) is linearly displaceable wherein the scanning device (3) defines a movement in a radial direction (5) and the specimen receiving device (2) defines a movement in a radial direction (6), and the relative movement between scanning device (3) and specimen receiving device (2) occurs in the radial direction (5, 6).
- [c3] 3. The apparatus as defined in Claim 2, characterized in that the scanning device (3) defines a further axis (7) of rotation wherein the scanning device (3) or the specimen receiving device (2) is arranged rotatably or pivotably about the axis (7).
- [c4] 4. The apparatus as defined in Claim 1, characterized in that the optical distance between the specimen (1) and scanning device (3) remains substantially constant during of a relative motion between the scanning device (3) and the specimen receiving device (2).
- [c5] 5. The apparatus as defined in Claim 1, characterized in that the specimen receiving device (2) defines a rotation speed of the specimen receiving device (2), and the rotation speed is dependent on the relative position between the specimen receiving device (2) and the scanning device (3).
- [c6] 6. The apparatus as defined in Claim 5, characterized in that the rotation speed is dependent on the detected data stream of the scanning device (3).
- [c7] 7. The apparatus as defined in Claim 1, characterized in that the specimen receiving device (2) has a large mass.

- [c8] 8.The apparatus as defined in Claim 1, characterized in that the specimen receiving device (2) receives a single, replaceable specimen vessel.
- [c9] 9.The apparatus as defined in Claim 1, characterized in that the specimen receiving device (2) receives a replaceable carousel insert (8).
- [c10] 10.The apparatus as defined in Claim 9, characterized in that the carousel insert (8) receives individual specimen holders (9).
- [c11] 11.The apparatus as defined in Claim 10, characterized in that the individual specimen holders (9) are positionable in a predefinable plane on the carousel insert (8) with retaining means (10).
- [c12] 12.The apparatus as defined in Claim 10, characterized in that the individual specimen holders (9) are positioned resiliently.
- [c13] 13.The apparatus as defined in Claims 1, characterized in that an autofocussing means is provided for maintaining the specimens in focus.
- [c14] 14.The apparatus as defined in Claim 13, characterized in that the autofocussing means maintains the surface of the rotating specimen receiving device (2) or of the specimen vessel or of the specimen holders (9) located in the carousel insert (8) always within a deviation in the direction of the optical axis (12) of the scanning device (3) of less than 20 μ m.
- [c15] 15.The apparatus as defined in Claim 1, characterized in that at least one laser beam is provided for scanning the specimens (1) and at least one detector (14) detects the light reflected from the specimens.
- [c16] 16.The apparatus as defined in Claim 15, characterized in that the laser beam scans in at least one direction.
- [c17] 17.The apparatus as defined in Claim 15, characterized in that the laser beam is stationary relative to the scanning device (3).
- [c18] 18.The apparatus as defined in Claim 15, characterized in that scanning is accomplished with laser light of different wavelengths.

- [c19] 19.The apparatus as defined in Claim 15, characterized in that the laser beam has an axial extent of the focus region in the specimen region of less than 40 μ m.
- [c20] 20.The apparatus as defined in Claims 15, characterized in that the laser beam has a lateral extent of the focus region in the specimen region in a range between 5 μ m and 200 μ m.
- [c21] 21.The apparatus as defined in Claims 15, characterized in that the laser beam defines an incidence angle on the surface of the specimen receiving device (2) or the specimen vessel or onto the specimen holders (9), wherein the incidence angle is greater or less than zero.
- [c22] 22.The apparatus as defined in Claim 14, characterized in that synchronization markers (19) are provided on the specimen receiving device (2) or the specimen vessel or the carousel insert (8).

[illegible]

Abstract of Disclosure

An apparatus for optical scanning of multiple specimens (1) and/or specimen regions, the specimens (1) being associated with a specimen receiving device (2) and being optically scannable by a scanning device (3), is easy to operate for data recording of many specimens in the shortest possible time, using simple and economical optical system components. The apparatus according to the present invention is characterized in that the specimen receiving device (2) is rotatable about an axis (4).

[illegible]

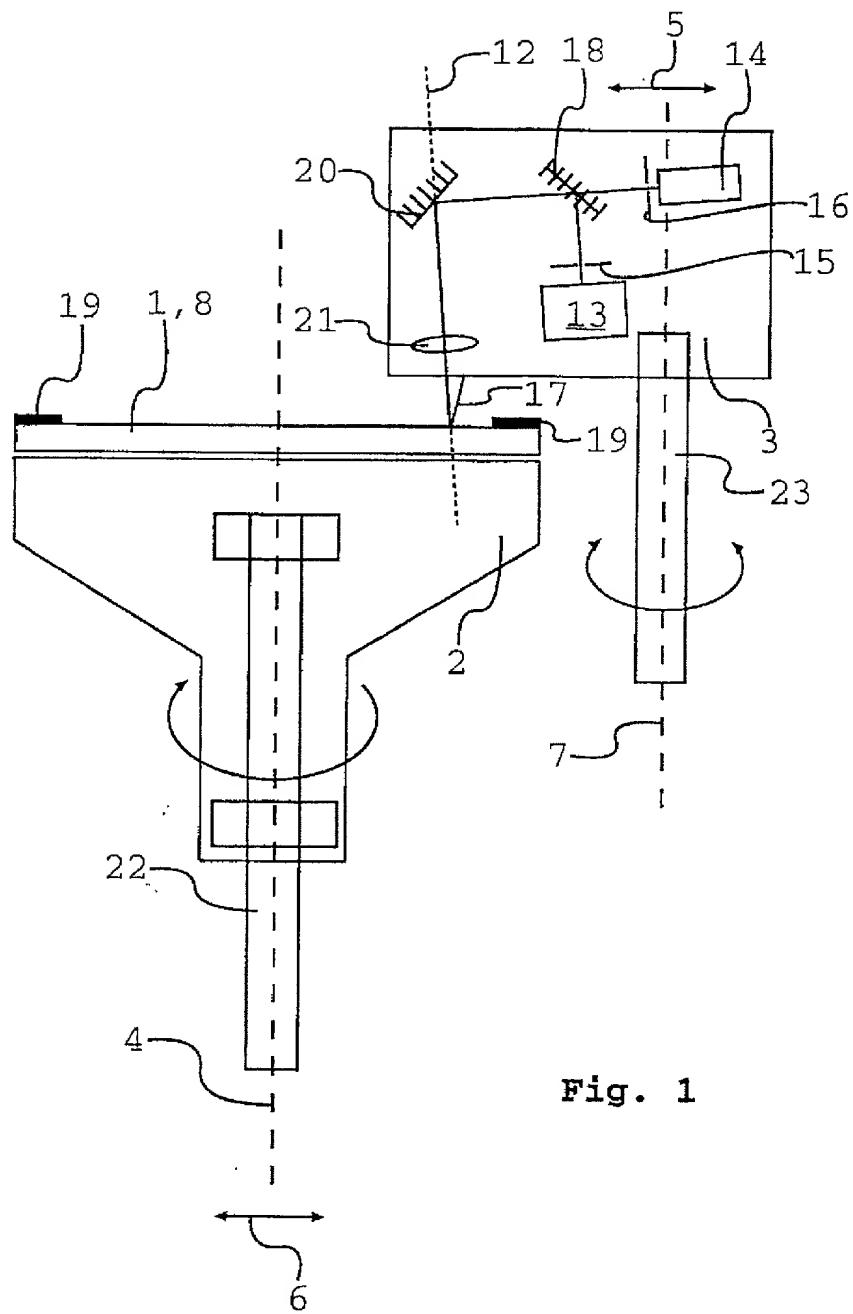
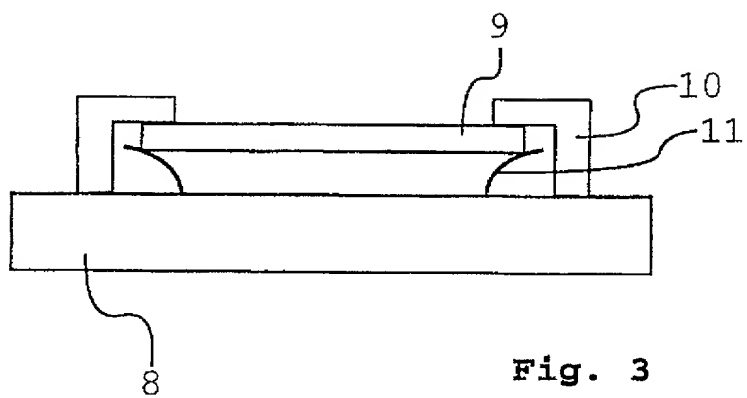


Fig. 1

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My residence, post-office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or a
original, first and joint inventor (if plural names are listed below) of the subject matter which
is claimed and for which a patent is sought on the invention entitled:

APPARATUS FOR OPTICAL SCANNING OF MULTIPLE SPECIMENS

the specification of which

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Country	Application Number	Date of Filing (month, day, year)	Priority Claimed Under 35 USC 119
Germany	DE 199 57 413.8	November 29, 1999	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No
			<input type="checkbox"/> Yes <input type="checkbox"/> No
			<input type="checkbox"/> Yes <input type="checkbox"/> No
			<input type="checkbox"/> Yes <input type="checkbox"/> No
			<input type="checkbox"/> Yes <input type="checkbox"/> No

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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment or both under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon. I hereby appoint:

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Maria M. Eliseeva	Reg. No. 43,328	Tram Anh T. Nguyen	Reg. No. 47,257
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William C. Geary III	Reg. No. 31,359	Scott D. Rothenberger	Reg. No. 41,277
Lisa J. Michaud	Reg. No. 44,238		

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